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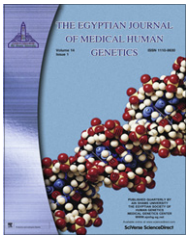

ORIGINAL ARTICLE

Lack of association between *TRAF1/C5* rs10818488 polymorphism and rheumatoid arthritis in Iranian population

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Abstract Rheumatoid arthritis (RA) is a multifactorial disorder related to the inflammatory response system with debilitating and painful conditions. Both genetic and environmental factors, with unknown etiology, play important roles in this disease pathogenesis. Recently, *TRAF1/C5* (*Tumor Necrosis Factor Receptor-Associated Factor 1/Complement Component 5*) polymorphism associated with increased risk for RA has been studied in different populations worldwide, and inconsistent results have been obtained. rs10818488 allele is located on *TRAF1/C5* intergenic region, and has been predicted to be functional. A total of 100 sex- and age-matched people including RA patients ($n = 50$) and healthy individuals ($n = 50$) from Iran have been entered in this study and genotyped for rs10818488 (A/G) polymorphism, using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). In our study, rs10818488 allele was not associated with risk for RA in Iranian population ($p > 0.05$, OR = 1.27, 95% CI = 0.72–2.23). Results revealed that this allele might be population-specific and not to be associated with their corresponding gene pool. However, further analyses are required to clarify other RA-associated markers in our community.

1. Introduction

Rheumatoid arthritis (RA) is a multifactorial disorder related to the inflammatory response system and characterized by progressive joint damage and increased mortality, with aetiological wide spectrum of phenotypes. Interactions between both environmental and genetic contributors confer heterogeneity

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of the disease [1–3]. Nowadays, our understanding about genetic determinants in RA has been improved by the study of many *HLA* and non-*HLA* genes. Among *HLA* genes, in all populations worldwide *HLA-DRB1* has a pivotal role in RA symptoms [4], whereas from non-*HLA* risk factors that have been found with a lower rate of association, *PTP22* [5,6], *TRAF1/C5* [7,8], *TNFAIP3* [5,9,10], and *STAT4* [11] have been demonstrated as promising RA-correlated markers [12,13]. *TRAF1/C5* region on chromosome 9q33-34, both of immune response-related genes, has been in recent years identified to impact on RA [7,14]. Significant depletion of complement components followed by elevated level of C5a in synovial fluid of RA patients [15] and resistance to arthritis in C5a-receptor deficient mice [16,17] as well as linkage studies showed the involvement of a complement system in RA pathogenesis [7,18,19]. TRAF1 is a critical adaptor in proinflamatory cytokine TNF-alpha signaling cascade as a RA-pathogenic effecter. In this cascade, stimulation of TNF-receptor 2 (TNFR2) induces TRAF2 degradation and inhibits the TNFR1-induced NF-KB activation. In contrast TRAF1 inhibits TRAF2 degradation; subsequently signal would be changed from apoptosis toward NF-KB activation and proinflamatory responses [20,21]. However, several population genetic studies also suggested that different single nucleotide polymorphisms (SNPs) at TRAF1/C5 flanking region has a critical role in RA, but different results have been obtained. rs10818488 in this region is predicted to be functional and may affect *TRAF1* and C5 expression [7]. Although, this variant is known as a RA-susceptibility, it is not consistent in all populations. Since no evidence of the relationship between TRAF1/C5 region polymorphisms (rs10818488 allele) and RA has been reported in populations from the west of Asia, we have decided to study rs10818488 allele and its relation with RA in Iranian populations.

2. Subjects and methods

2.1. Study population

The subjects enrolled in this study comprised 50 patients with RA and 50 healthy controls from unrelated families living in the province of Kermanshah (west of Iran). The diagnosis of RA was made according to the American Rheumatism Association 1987 revised criteria [22]. All participants gave written informed consent and people with unrelated origin were excluded.

2.2. Genotyping for rs10818488

Genomic DNA samples of all individuals were isolated from whole blood collected with anticoagulant (EDTA, 15% w/v), using a 'salting out' method [23]. The forward primer 5′-GCA GCA GCA GAA CTA CGT GA 3′ and the reverse primer 5′-GCT TGC TGT TGA AAT CCT GAA GG-3′ were used to amplify a 226 bp-intergenic region of the *TRAF1/C5* locus. The amplification was carried out using the *Taq* DNA polymerase (Fermentas). Polymerase chain reaction (PCR) products were analyzed through electrophoresis on 1.5% agarose gel in reference to a molecular weight marker.

Genotyping for the rs10818488 (A/G) polymorphism was performed by restriction analysis according to Zervou et al. [25]. Genotypes were scored blindly and analysis of all samples was repeated twice for checking the accuracy of the results.

2.3. Statistical analysis

In this case-control study, the variant under investigation was evaluated for deviation from Hardy–Weinberg equilibrium by comparing observed and expected genotype frequencies by means of Chi-square test in the control groups. The statistical difference in genotype distribution and allele frequencies in both control and case subjects was assessed by using standard 2×2 test. Odds ratios (ORs) and confidence intervals (CIs) were calculated and a p value of 0.05 was determined as significant.

3. Results

The RA study group ($n = 50$) and unrelated healthy controls ($n = 50$) were of similar sex and age and consisted of 40 women and 10 men with 58 ± 5 years (Mean ± SD). Allele and genotype frequencies of the analyzed samples of the *TRAF1/C5* rs10818488 G/A polymorphism are depicted in the table below.

Study groups	Mutant Allele A (%)	Wild type Allele G (%)	*P* Value	Odd ratio (95% CI)
Allele Frequency				
RA patients ($n = 100$)	46(46)	54(54)	> 0.05	1.27 (0.72–2.23)
Controls ($n = 100$)	40(40)	60(60)		
Genotype frequency	G/A or A/A	G/G		
RA patients ($n = 50$)	44 (88)	6 (12)	>0.05	1.83 (0.61–5.5)
Controls ($n = 50$)	40 (80)	10 (20)		

Deviation from Hardy–Weinberg equilibrium was not found with regard to genotypes distribution in the control group ($p > 0.05$). We observed that the G/A or A/A genotypes were not common in RA patients (88%) as compared with control individuals (80%) and the difference was not statistically significant ($p > 0.05$). The observed difference was calculated with a 2×2 χ^2 test. So, it cannot be considered that there is a relationship between mutant genotypes (G/A or A/A) and Iranian patients with RA (OR = 1.83, 95% CI = 0.61–5.5). Patients with RA also did not commonly show the A allele (46%) compared to controls (40%) ($p > 0.05$, OR = 1.27, 95% CI = 0.72–2.23). Therefore, it can be regarded that there is no association between the mutant allele and RA in patients of Iranian origin. Gel pictures depicted below show G/G, G/A (Fig. 1.) and A/A (Fig. 2.) genotypes in our study.

4. Discussion

Relationships between different candidate genes and RA disease in different ethnic groups have been recently evaluated in several studies. Candidate gene approach revealed that the

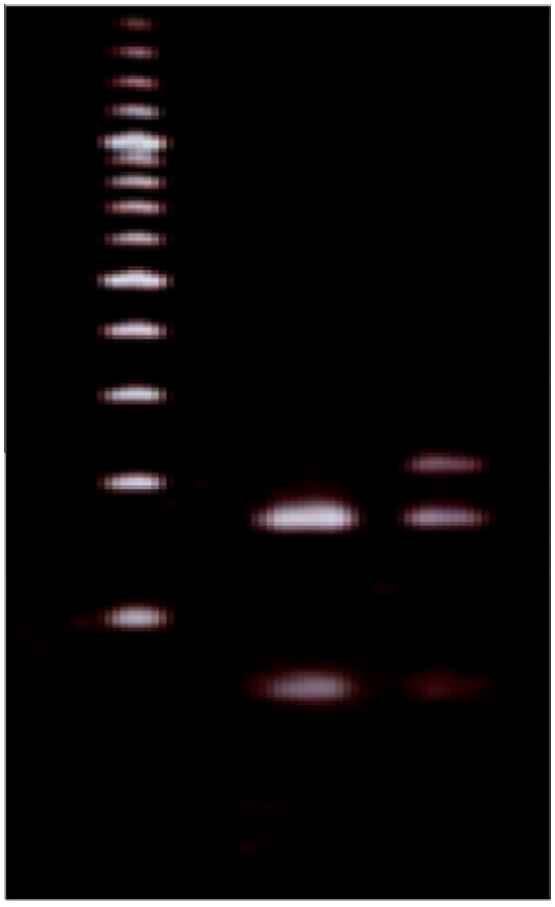

Figure 1 G/G and G/A genotypes are depicted; Lane 1: 100-bp Ladder, Lane 2: G/G (G allele digested into 169- and 57-bp fragments), Lane 3: G/A (digested G and undigested A allele).

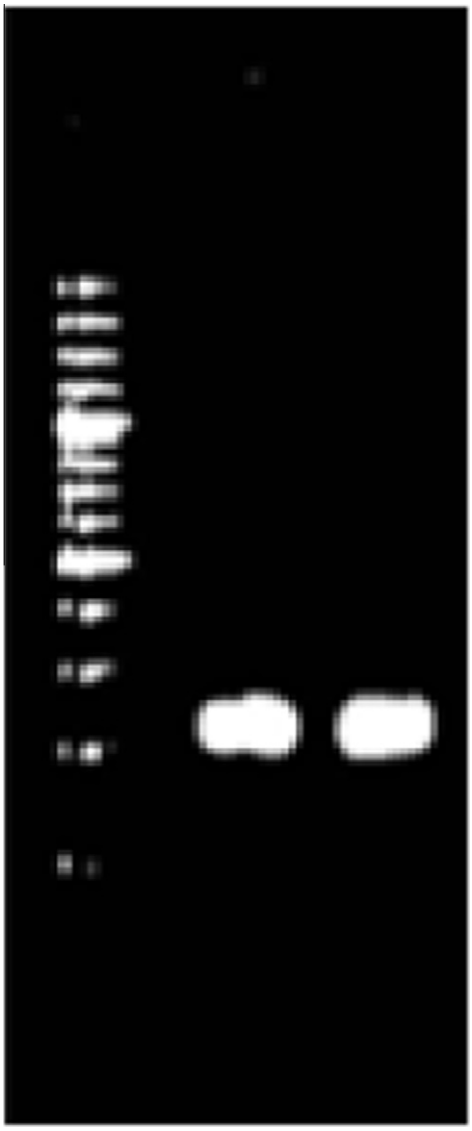

Figure 2 A/A genotype is depicted; Lane 1: 100-bp Ladder, Lane 2 and 3: undigested A allele (226-bp fragment).

TRAF1/C5 rs10818488 (A/G) polymorphism could affect susceptibility to RA in Dutch [7] and Spanish (Kureeman et al., data not reported) populations. Although, a meta-analysis [24] and association studies in Creten [25] Egyptian ethnicities [26] and western European white individuals from ''trio'' families [27] confirmed this finding. However, similar observations were not found in Swedish [5] and Colombian [28] populations, these inconsistencies were also reported in Asians. Mutated A allele of rs10818488 is not associated with increasing risk of RA in Korean population [29] though; Nishimoto et al. observed that wild type (G) allele of rs10818488 was modestly associated with RA in Japanese [30]. Interestingly, other *TRAF1/C5* polymorphic alleles such as rs3761847 have been shown to be significantly associated with RA in Korean [29] and Han Chinese [31] Asians. Our study provides evidence that there is no relation between rs10818488 and RA in Iranian population. Therefore, rs10818488 might not be a genius allele related to RA in Asians and also, there are distinct population specific differences in the prevalence of this allele. The point should not to be forgotten that the Iranian population is composed of different ethnic groups, so, identifying alleles that have lower frequency in this population is hardly possible. It must be flagged out that the gene pool in our studied population may specifically have other RA-susceptibility alleles because of migration, recombination and so on. Thus, further investigations are needed to prove our study.

These challenging findings and increased *TRAF1* expression level in phorbol myristate acetate-stimulated lymphoblastoid cell lines [30], in most recent studies, may reflect epigenetic effects of rs10818488 allele in different populations. This allele is located in *TRAF1/C5* intergenic region and predicted to be a putative binding site for transcription factor EP300 [7]. EP300 plays important roles in vast biological processes, including cell proliferation and differentiation [32]. Moreover, rs10818488 may regulate the expression of both neighbour genes, *TRAF1* and *C5* that are involved in proinflamatory TNF alpha signaling cascade, and as a result influencing RA pathogenesis.

5. Conclusion

Although, many RA-associated genes (about 31 genes) have been reported by now [5,7–11,33–35], many efforts remained

to be attempted to make a gene map for these markers. At the end we cannot turn a blind eye on environmental factors in RA development and progression.

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